

Investigation 7: Cell Division

Part A: Mitosis

Background

One of the characteristics of living things is the ability to replicate and pass on genetic information to the next generation. Cell division in individual bacteria and archaea usually occurs by binary fission. Mitochondria and chloroplasts also replicate by binary fission, which is evidence of the evolutionary relationship between these organelles and prokaryotes.

Cell division in eukaryotes is more complex. It requires the cell to manage a complicated process of duplicating the nucleus, other organelles, and multiple chromosomes. This process, called the cell cycle, is divided into three parts: interphase, mitosis, and cytokinesis (Figure 1). In the first growth phase (G_1), the cell grows and prepares to duplicate its DNA. In the synthesis phase (S), the chromosomes are replicated. In the second growth phase (G_2), the cell prepares to divide. In mitosis, the duplicated chromosomes are separated into two nuclei. In most cases, mitosis is followed by cytokinesis, when the cytoplasm divides and organelles separate into daughter cells. This type of cell division is asexual and is important for growth, renewal, and repair of multicellular organisms.

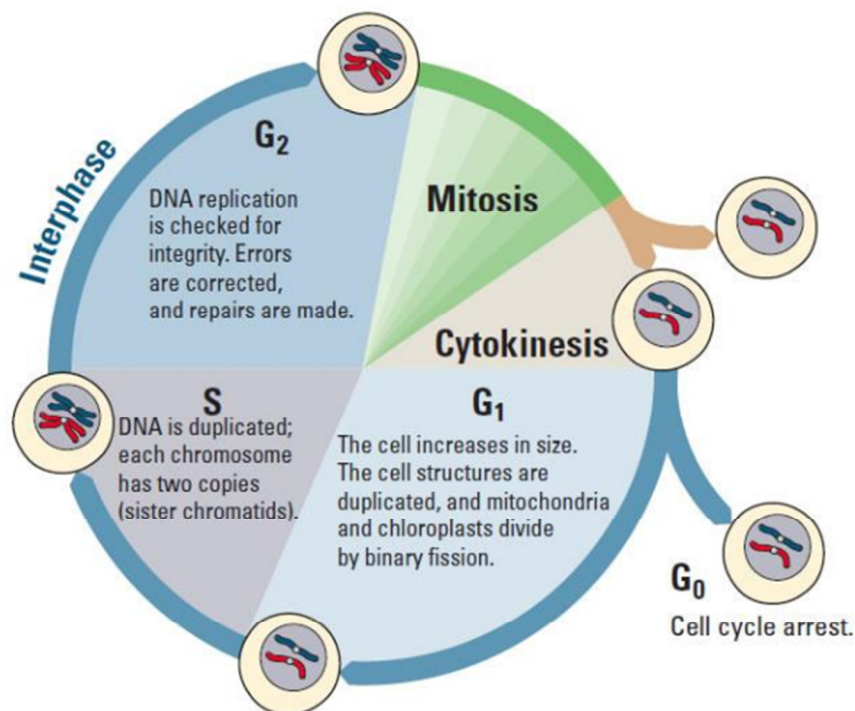


Figure 1. Major Phases of the Cell Cycle

Name _____

Cell division is tightly controlled by complexes made of several specific proteins. These complexes contain enzymes called cyclin-dependent kinases (CDKs), which turn on or off the various processes that take place in cell division. CDK partners with a family of proteins called cyclins. One such complex is mitosis-promoting factor (MPF), sometimes called maturation-promoting factor, which contains cyclin A or B and cyclin-dependent kinase (CDK). (See Figure 2a) CDK is activated when it is bound to cyclin, interacting with various other proteins that, in this case, allow the cell to proceed from G_2 into mitosis. The levels of cyclin change during the cell cycle (Figure 2b). In most cases, cytokinesis follows mitosis.

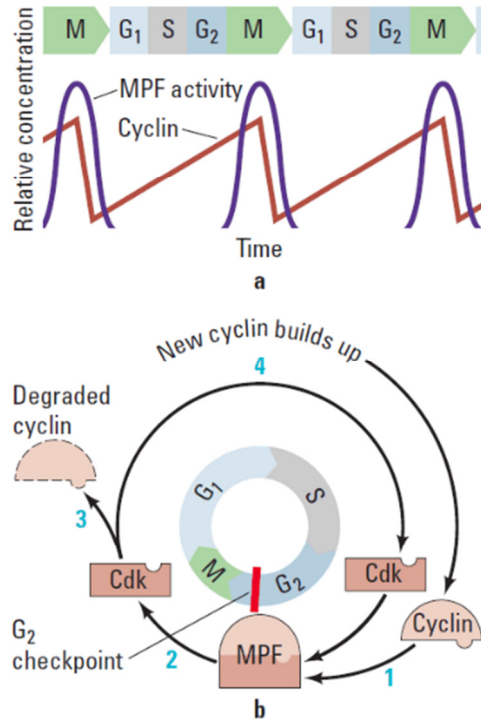


Figure 2. MPF Production During the Cell Cycle

As shown in Figure 3, different CDKs are produced during the phases. The cyclins determine which processes in cell division are turned on or off and in what order by CDK. As each cyclin is turned on or off, CDK causes the cell to progress through the stages in the cell cycle.

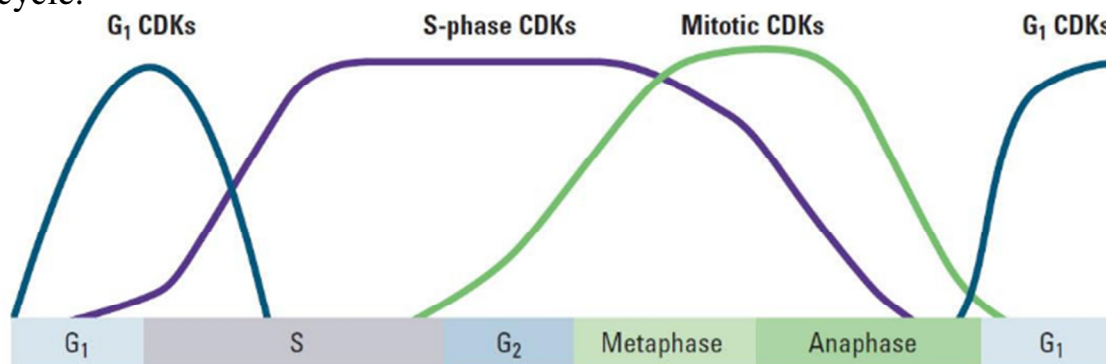


Figure 3. Levels of CDKs During the Cell Cycle

Cyclins and CDKs do not allow the cell to progress through its cycle automatically. There are three checkpoints a cell must pass through: the G₁ checkpoint, G₂ checkpoint, and the M-spindle checkpoint (Figure 4). At each of the checkpoints, the cell checks that it has completed all of the tasks needed and is ready to proceed to the next step in its cycle. Cells pass the G₁ checkpoint when they are stimulated by appropriate external growth factors; for example, platelet-derived growth factor (PDGF) stimulates cells near a wound to divide so that they can repair the injury. The G₂ checkpoint checks for damage after DNA is replicated, and if there is damage, it prevents the cell from going into mitosis. The M-spindle (metaphase) checkpoint assures that the mitotic spindles or microtubules are properly attached to the kinetochores (anchor sites on the chromosomes). If the spindles are not anchored properly, the cell does not continue on through mitosis. The cell cycle is regulated very precisely. Mutations in cell cycle genes that interfere with proper cell cycle control are found very often in cancer cells.

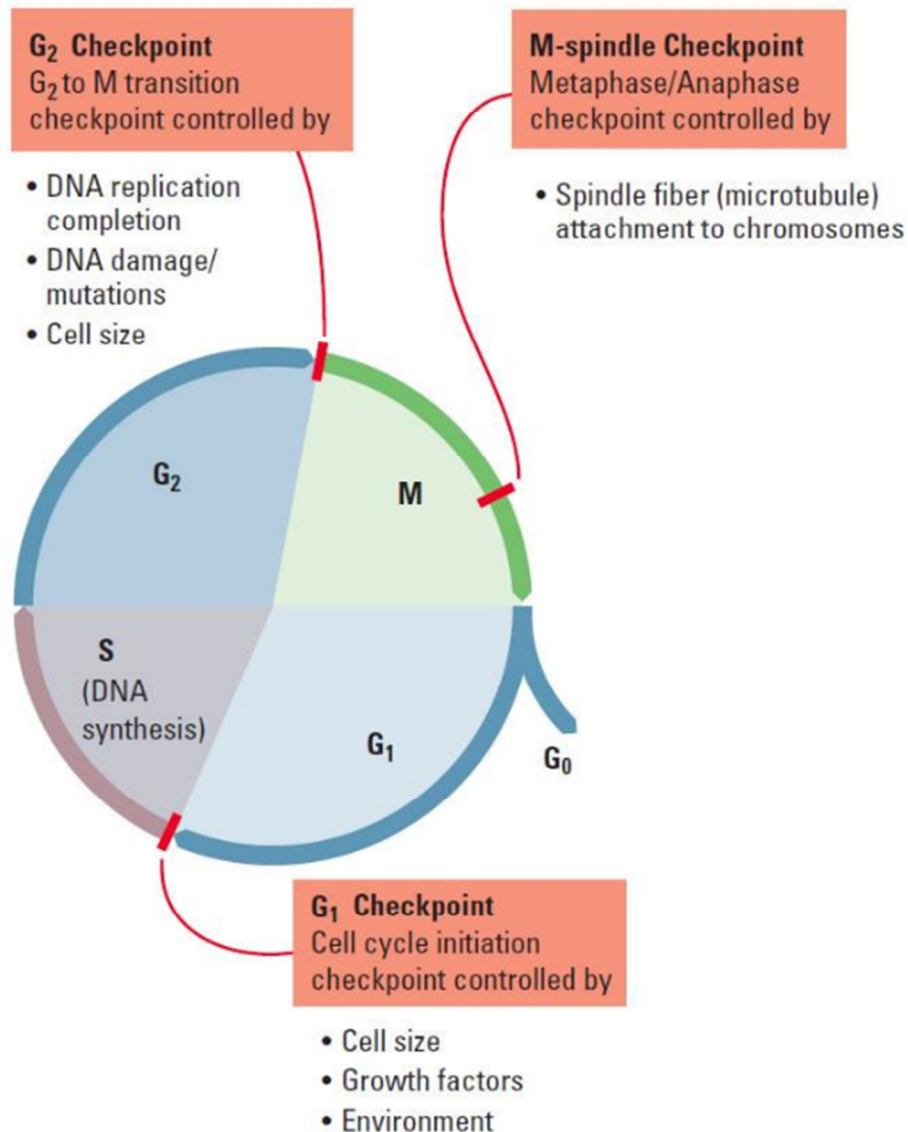


Figure 4. Diagram of the Cell Cycle Indicating Key Checkpoints

Name _____

Figure 5 illustrates how the chromosomes move during mitosis. It is important to see how the duplicated chromosomes align, separate, and move into new cells. Figure 6 is an onion root tip showing various stages of the cell cycle.

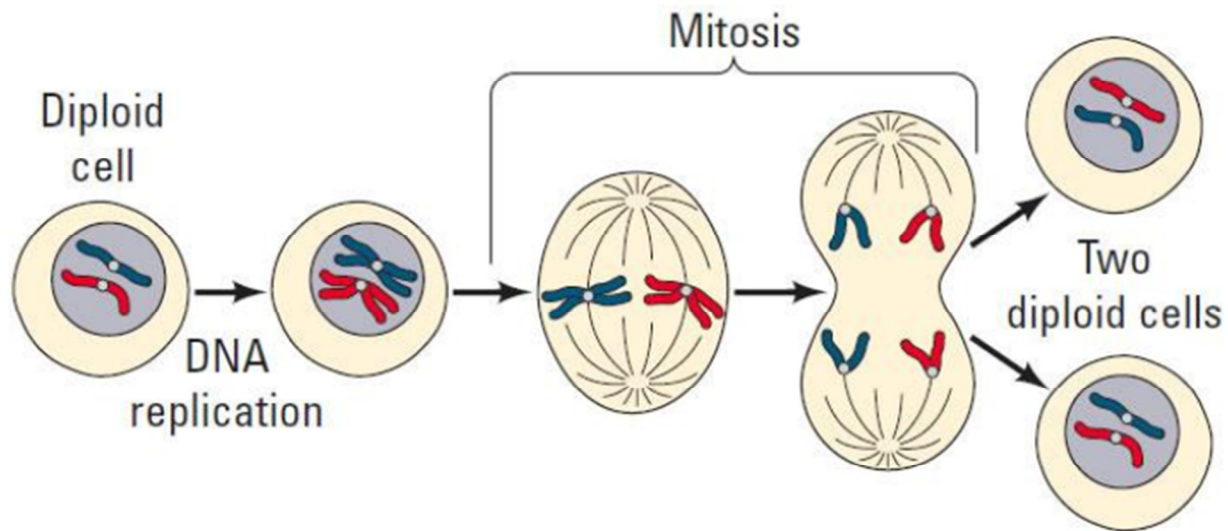


Figure 5: Mitotic Cell Division Emphasizing Chromosome Movement

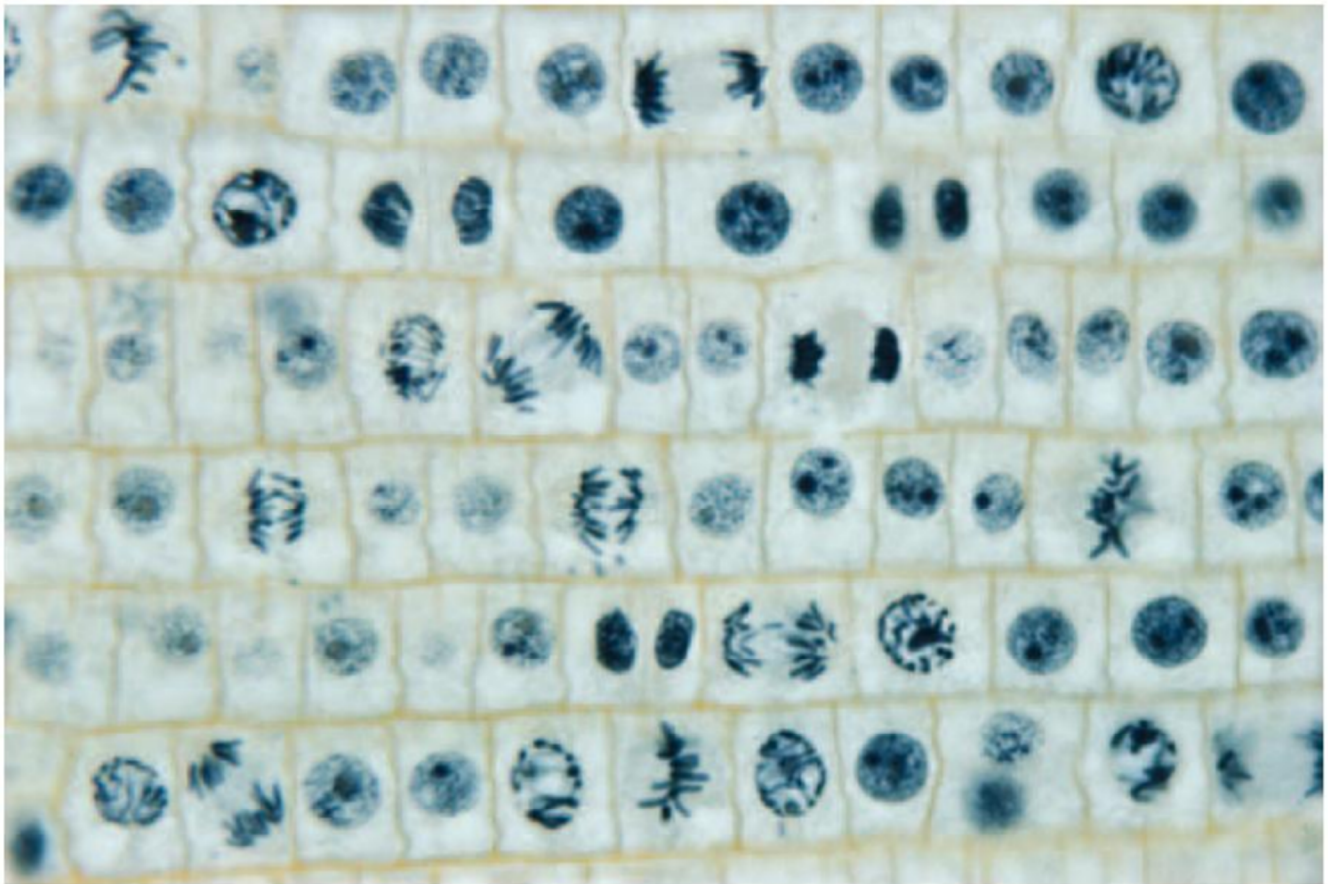


Figure 6: Onion Root Tip

Environmental Effects on Mitosis

The health of a plant or animal depends upon both biotic and abiotic factors. Space, water, nutrients, and light are abiotic factors that can affect the height of a plant's stem, its number of leaves, and the number and length of the roots. Many biotic factors also affect plant growth. A classic example of a beneficial biotic effect is the mutualistic relationship between legumes (beans, peas, clover, and alfalfa) and the nitrogen-fixing bacterium, rhizobia. *Rhizobia* (singular = rhizobium), lives in nodules on the roots of beans and other plants. Chemicals released by the plant cause the bacterium to migrate toward the plant roots. Entry of the bacterium into the root causes a cascade of cell signals. That area of the root enters a phase of rapid cell division producing a nodule where the bacteria flourish.

However, not all biotic interactions benefit a plant. Parasitic interactions may harm a plant by increasing mitosis. For example, the plant pathogen *Agrobacterium tumefaciens* (now called *Rhizobium radiobactor*) causes plant cancer or galls. By triggering a plant to undergo rapid cell division, the pathogen forces the plant to expend more energy in that location and not in the other roots, stems, and leaves. This weakens the plant and may cause death. *A. tumefaciens* enters the plant through a wound and infects cells by inserting a plasmid into the cell. The plasmid inserts into the DNA and causes several important genes to be transcribed. One gene codes for the plant hormone indole-3-acetic acid, IAA. IAA is a plant hormone that triggers cell division and has been used commercially as a rooting compound for many years.

Scientists reported that a fungal pathogen may also negatively affect the growth of soybeans (*Glycine max*). The soybean growth was decreased during three years of high rainfall. The soybean roots were poorly developed. *R. anaerobis* is another fungus that also is a plant pathogen that grows in the soil. *R. anaerobis* releases the protein lectin. A lectin-like protein was found in soil surrounding the affected soybean roots. Lectins accelerate mitosis in some root apical meristems (growth regions of plants—similar to stem cells in animals); however, in many instances, rapid cell division weakens plant tissues.

We are using onions instead of soybeans since onion root tips are more easily grown and studied. Onions germinate easily without soil so the chemicals provided to the plant can be easily controlled. Onion root tips also grow quickly and are only a few cells thick. A stain is used to dye condensed chromosomes like those undergoing mitosis a very dark color. By viewing the onion root tip using a light microscope it is easy to determine if a particular cell is in interphase or mitosis. Figure 7 shows the anatomy of an onion root tip. Note that cell division occurs only in the meristem region, not in the other regions of the root tip. Recall also that 90% of the time a cell in this region will be in interphase, since mitosis typically makes up only 10% of a full cell cycle.

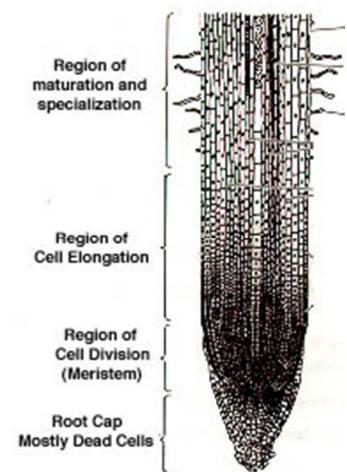


Figure 7. Apical meristem

Name _____

Pre-Lab Questions

1. Practice identifying cells in various stages of the cell cycle by counting the cells in Figure 6. Record your results in Table 1 below.

Table 1. Count of cells in the various stages of the cell cycle

Phase	Interphase	Prophase	Metaphase	Anaphase	Telophase	Total
Number of Cells						
Percent of Cells in Phase						

2. Students conducted an experiment to determine if caffeine influences cell division. They treated some onion roots with coffee and others with distilled water as a control. Use their data found in Table 2 to determine if there is a statistical difference between the two treatments based on the number of cells in mitosis.

Table 2. Comparison of dividing and nondividing cells in control vs treated group

	Interphase	Mitosis	Total
Control group	60	40	100
Coffee treated group	90	110	200

- Write your null hypothesis
 -
 -
- Write your alternative hypothesis
 -
 -
- Formalize your thinking by making a contingency box
 - Use coffee treated group as observed results
 - To calculate expected results:
 - Determine the percentage of cells in interphase vs mitosis in the control group
 - Multiply the percentages by the total number of cells in the coffee treated group

Name _____

- Calculate your chi-square value
 - Show your work:

$$\chi^2 = \sum \frac{(\text{Observed Value} - \text{Expected Value})^2}{(\text{Expected Value})}$$

- Chi-square value =

- Determine the critical chi-square value (use a p-value of 0.05)

p value	Degrees of Freedom							
	1	2	3	4	5	6	7	8
0.05	3.84	5.99	7.82	9.49	11.07	12.59	14.07	15.51
0.01	6.64	9.21	11.34	13.28	15.09	16.81	18.48	20.09

- Degrees of freedom =
 - Critical chi-square value =
- Draw your conclusion
 - Our calculated chi-square value is _____ than our critical chi-square value
 - Therefore, we _____ our null hypothesis which means
 -
 -

Procedure

1. Count the number of cells in each phase of the cell cycle for Treatment A and enter your data in Table 3. Be sure to also determine the total number of cells in mitosis as well as the total number of cells counted overall.
2. Repeat for at least 2 more samples of Treatment A
3. Repeat steps 1 and 2 for Treatment B using Table 4
4. Compare the number of cells from each treatment in interphase and in mitosis. Predict which cells were treated with the control vs which were treated with lectin and CONFIRM with your teacher (record confirmed treatment above each table)
5. Determine if the fungal pathogen lectin significantly increased the number of cells in mitosis by using a chi-square test to statistically analyze the data.

Name _____

Results

Table 3. Onion Root Tip Cell Phase Data; Treatment Group A: _____

#	Number of Cells						
	Prophase	Metaphase	Anaphase	Telophase	Mitosis	Interphase	Total
1							
2							
3							
Total							

Table 4. Onion Root Tip Cell Phase Data; Treatment Group B: _____

#	Number of Cells						
	Prophase	Metaphase	Anaphase	Telophase	Mitosis	Interphase	Total
1							
2							
3							
Total							

Analysis

- Write your null hypothesis
 -
 -
- Write your alternative hypothesis
 -
 -
- Formalize your thinking by making a contingency box
 - Use lectin treated group as observed results
 - To calculate expected results:
 - Determine the percentage of cells in interphase vs mitosis in the control group
 - Multiply the percentages by the total number of cells in the lectin treated group

Name _____

- Calculate your chi-square value
 - Show your work:

$$\chi^2 = \sum \frac{(\text{Observed Value} - \text{Expected Value})^2}{(\text{Expected Value})}$$

○ Chi-square value =

- Determine the critical chi-square value (use a p-value of 0.05)

p value	Degrees of Freedom							
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- Degrees of freedom =
- Critical chi-square value =
- Our calculated chi-square value is _____ than our critical chi-square value

Conclusion

- Did you accept or reject your null hypothesis? Explain what this means (use complete sentences)